PROTEIN PURIFICATION

This application is a continuation of U.S. application Ser. No. 12/418,905 filed Apr. 6, 2009 (now abandoned), which is a continuation of U.S. application Ser. No. 11/398,447, filed Apr. 5, 2006, (now U.S. Pat. No. 7,531,645 issued May 12, 2009) which is a continuation of and claims priority under 35 U.S.C. §120 to U.S. patent application Ser. No. 10/949,683, filed Sep. 24, 2004, (now U.S. Pat. No. 7,074,404 issued Jul. 11, 2006), which is a continuation of and which claims priority to under 35 USC §120 to U.S. application Ser. No. 10/253,366 filed Sep. 24, 2002 (now abandoned), which claims priority to U.S. application Ser. No. 09/304,465 filed May 3, 1999 (now U.S. Pat. No. 6,489,447 issued Dec. 3, 2002), which claims priority under 35 U.S.C. §119 to provisional application No. 60/084,459 filed May 6, 1998, the entire disclosures of which are hereby incorporated by reference.

BACKGROUND OF THE INVENTION

1. Field of the Invention

This invention relates generally to protein purification. In particular, the invention relates to a method for purifying a polypeptide (e.g. an antibody) from a composition comprising the polypeptide and at least one contaminant using the method of ion exchange chromatography.

2. Description of Related Art

The large-scale, economic purification of proteins is increasingly an important problem for the biotechnology industry. Generally, proteins are produced by cell culture, using either mammalian or bacterial cell lines engineered to produce the protein of interest by insertion of a recombinant plasmid containing the gene for that protein. Since the cell lines used are living organisms, they must be fed with a complex growth medium, containing sugars, amino acids, and growth factors, usually supplied from preparations of animal serum. Separation of the desired protein from the mixture of compounds fed to the cells and from the by-products of the cells themselves to a purity sufficient for use as a human therapeutic poses a formidable challenge.

Procedures for purification of proteins from cell debris initially depend on the site of expression of the protein. Some proteins can be caused to be secreted directly from the cell into the surrounding growth media; others are made intracellularly. For the latter proteins, the first step of a purification process involves lysis of the cell, which can be done by a variety of methods, including mechanical shear, osmotic shock, or enzymatic treatments. Such disruption releases the entire contents of the cell into the homogenate, and in addition produces subcellular fragments that are difficult to remove due to their small size. These are generally removed by differential centrifugation or by filtration. The same problem arises, although on a smaller scale, with directly secreted proteins due to the natural death of cells and release of intracellular host cell proteins in the course of the protein production run.

Once a clarified solution containing the protein of interest has been obtained, its separation from the other proteins produced by the cell is usually attempted using a combination of different chromatography techniques. These techniques separate mixtures of proteins on the basis of their charge, degree of hydrophobicity, or size. Several different chromatography resins are available for each of these techniques, allowing accurate tailoring of the purification scheme to the particular protein involved. The essence of each of these separation methods is that proteins can be caused either to move at different rates down a long column, achieving a physical separation that increases as they pass further down the column, or to adhere selectively to the separation medium, being then differentially eluted by different solvents. In some cases, the desired protein is separated from impurities when the impurities specifically adhere to the column, and the protein of interest does not, that is, the protein of interest is present in the "flow-through".

Ion exchange chromatography is a chromatographic technique that is commonly used for the purification of proteins. In ion exchange chromatography, charged patches on the surface of the solute are attracted by opposite charges attached to a chromatography matrix, provided the ionic strength of the surrounding buffer is low. Elution is generally achieved by increasing the ionic strength (i.e. conductivity) of the buffer to compete with the solute for the charged sites of the ion exchange matrix. Changing the pH and thereby altering the charge of the solute is another way to achieve elution of the solute. The change in conductivity or pH may be gradual (gradient elution) or stepwise (step elution). In the past, these changes have been progressive; i.e., the pH or conductivity is increased or decreased in a single direction.

SUMMARY OF THE INVENTION

The present invention provides an ion exchange chromatographic method wherein a polypeptide of interest is bound to the ion exchange material at an initial conductivity or pH and then the ion exchange material is washed with an intermediate buffer at a different conductivity or pH, or both. At a specific point following this intermediate wash, and contrary to ion exchange chromatography standard practice, the ion exchange material is washed with a wash buffer where the change in conductivity or pH, or both, from the intermediate buffer to the wash buffer is in an opposite direction to the change in conductivity or pH, or both, achieved in the previous steps. Only after washing with the wash buffer, is the ion exchange material prepared for the polypeptide molecule of interest to be eluted by the application of the elution buffer having a conductivity or pH, or both, which differ from the conductivity or pH, or both, of the buffers used in previous steps.

This novel approach to ion exchange chromatography is particularly useful in situations where a product molecule must be separated from a very closely related contaminant molecule at full manufacturing scale, where both purity and high recovery of polypeptide product are desired.

Accordingly, the invention provides a method for purifying a polypeptide from a composition comprising the polypeptide and a contaminant, which method comprises the following steps performed sequentially:

(a) binding the polypeptide to an ion exchange material using a loading buffer, wherein the loading buffer is at a first conductivity and pH;

(b) washing the ion exchange material with an intermediate buffer at a second conductivity and/or pH so as to elute the contaminant from the ion exchange material;

(c) washing the ion exchange material with a wash buffer which is at a third conductivity and/or pH, wherein the change in conductivity and/or pH from the intermediate buffer to the wash buffer is in an opposite direction to the change in conductivity and/or pH from the loading buffer to the intermediate buffer; and

(d) washing the ion exchange material with an elution buffer at a fourth conductivity and/or pH so as to elute the